REMARKS

Applicants have amended the claims to more particularly describe one embodiment of the present invention. More particularly, applicants have canceled the non-elected subject matter of claims 9-35. In addition, claim 1 has been amended to incorporate the limitations of original claim 21 specifying the specific bacterial isolates (by reference to their ATCC accession number) used in the claimed method. Additional support for claim 1 is found on page 33, lines 15-18. As disclosed in the present specification these specific bacterial isolates have been found to exhibit unique characteristics making them particularly well suited for the claimed method. Accordingly, with the amendment to claim 1, claims 4-8 have been rendered redundant and therefore have been canceled.

Claims 36-41 are new and are directed to further embodiments of the present invention. Support for the new claims is found throughout the specification including for example, on page 29, lines 15-20 for claims 36-38; and in tables 1-12, page 33, lines 15-24 and the original claims, for new claims 39-41.

Claim 1 stands rejected under 35 USC 102(b) as being anticipated by, or in the alternative, under 35 USC 103 as being obvious over the teachings of Daeschel et al. Applicants respectfully submit that claim 1 as amended is neither taught nor suggested by Daeschel et al.

Applicants respectfully submit the claimed invention requires the application of specific bacterial isolates that are neither disclosed nor suggested by Daeschel et al. As detailed in the data provided on pages 11-32 of the present application the specific referenced bacterial isolates were discovered to have advantageous properties relative to the vast majority of similar strains. In particular, the identified isolates can grow and produce their anti-*Listeria monocytogenes* properties over a broad range of temperatures (3-37°C, including refrigeration temperatures which is unusual) and environments (aerobic, anaerobic, and poor nutrition).

Daeschel et al. is primarily directed to the use of a liquid solution of bacteriocin, nisin, for food contact use such as in food preparation (kitchen surface) or packaging. This methodology represents a time-limited surface treatment on food contact areas because the activity of the enzyme is greatly influenced by the treated surface environment, such as pH, temperature, iron concentration and other factors. As noted by the Examiner the reference also discloses that one way to "apply" the bacteriocin is to prepare a biofilm of a bacteriocin

producing bacteria on the surface. However, Daeschel fails to teach, or provide any guidance of, how to prepare a bacteriocin producing bacteria that is capable of growing on surfaces that have poor nutrition sources and are kept at relatively low temperatures (e.g., 3-29°C). Such conditions are typical of food processing facility surfaces. The claimed invention, as amended, is limited to the use of live bacteria which have adapted to the various temperatures and environments (aerobic, anaerobic and poor nutrition) on food processing facility surfaces (including for example, floor drains) where pathogens, like *Listeria monocytogenes*, are difficult to remove and kill by commercially available treatments.

Daeschel et al simply fail to teach or suggest a method that uses live bacterial strains that have the ability to inhibit pathogenic organisms such as *Listeria monocytogenes* under the conditions commonly associated with food processing facility surfaces. Accordingly, applications respectfully submit Daeschel et al fails to teach or suggest the claimed invention, and applications request the withdrawal of the rejection of claim 1 as being anticipated, or in the alternative, obvious over that reference.

Claims 2-8 stand rejected under 35 USC 103 as being obvious over the teachings of Daeschel et al in view of Sulzer et al and DeLoach et al. Claims 3-8 have been canceled rendering the rejection as to those specific claims moot. However, applicants respectfully traverse this rejection as it applies the claim 2 and new claims 36-41, that remain pending in the application.

The deficiencies of the Daeschel et al reference have been described in the preceding section. The secondary references fail to supplement the deficiencies of the Daeschel et al reference in terms of the claimed invention. In particular applicants note that the relevant characteristics of lactic acid bacteria, including the *Enterococcus faecalis* and *Lactobacillus paracasei* strains referenced by the Examiner from the Sulzer et al. article, is that they require anaerobic (no oxygen) growth with good nutrition (including food such as Camembert cheese) and a suitable temperature (e.g., 30-33°C) for the production of a bacteriocin like nisin. However, contrary to previously characterized lactic acid bacteria, the isolates specifically referenced in amended claim 1, are not as fastidious as most lactic acid bacterial stains, including those described by Daeschel et al.

The four lactic acid bacteria isolates identified in amended claim 1 can grow and produce their anti-*Listeria monocytogenes* properties at a broad range of temperatures (3-37°C, including, advantageously, refrigeration temperatures of 4°C which is highly unusual) and environments (aerobic, anaerobic, and poor nutrition). The identified lactic acid bacteria

of the claimed method can also penetrate, adhere to, grow and produce metabolite(s) that kill or inhibit the growth of *L. monocytogenes* when colonized as biofilms on floor drain surfaces. Only these four isolates, which were deposited at ATCC as strain numbers PTA-4758, PTA-4759, PTA-4760, and PTA-4761 demonstrated these abilities in field trials.

Furthermore, the studies reported in U.S. Patents (No. 5,451,369 and No. 5,308,615), and in Sulzer et al., as well as the other references listed in the prior art references are laboratory-based results. None of the results obtained from these studies were repeated or confirmed under real-world conditions such as in food processing environments or kitchens. Accordingly, there is no teaching in any of the cited references that would demonstrate to one of ordinary skill in the art that the lactic acid bacteria they describe can control *Listeria* in floor drains or under similar commercially relevant conditions. One of ordinary skill in the art would have no reasonable expectation of successfully identifying bacteria that can effectively function to inhibit the growth of a pathogenic organism under the conditions present at food processing facilities, including the conditions found on floor drain surfaces.

The present application provides extensive data (Tables 1-12) based on field trials that reveal the claimed method is effective under real-world conditions, i.e., food processing facility surfaces. The claimed method using the specified lactic acid bacteria reduces/eliminates *Listeria* species and/or *Listeria monocytogenes* in floor drains in which temperatures ranged widely, from 3° to 26°C. The cited prior art merely reports that various lactic acid bacteria can exhibit inhibitory ability against different microorganisms, including *Listeria monocytogenes*, in agar plates, food or biofilms under the best of conditions for such inhibitory activity (ie., using selective medium and relatively high temperatures of about 35°C). No studies in any of the prior art were tested in a tube assay or floor drains (a critical evaluation approach for commercial application) and performed at low temperatures at which lactic acid bacteria typically do not grow. For example, applicants note that the lactic acid bacteria disclosed in U.S. Patent No. 5,308,615 are anaerobic bacteria, their isolates cannot survive in aerobic condition, such as those found on food processing facility surfaces. In contrast, the lactic bacteria, ATCC PTA-4758, PTA-4759, PTA-4760, and PTA-4761 used in the claimed method can grow and produce metabolite(s) under aerobic conditions.

As of today only the bacterial isolates disclosed in the present application and deposited in the ATCC have been validated to demonstrate commercial application in controlling *Listeria* species and/or *Listeria monocytogenes* in floor drains of food processing plants, which are important sources of Listeria contamination in food processing facilities.

As disclosed above the lactic acid bacterial isolates identified in amended claim 1 have unique characteristics that enable them to survive and grow in a nutritionally unfavorable environment, such as floor drains in food processing facilities (most lactic acid bacteria require fastidious nutritional conditions for survival and growth). The disclosed bacteria also exhibit their inhibitory effects at a broad range of temperatures (4°, 8°, 15°, and 37°C in agar plates, tubes, and biofilms), and more importantly at temperatures of 3-26°C (see page 29-32 of the present specification) that are typically found in floor drains.

The prior art references simply fail to provide an enabling disclosure for isolating the specific, unique probiotic isolates that applicants have deposited under ATCC Accession Numbers, PTA-4758, PTA-4759, PTA-4760, and PTA-4761 that have the necessary growth characteristics to be effective inhibitors of pathogenic bacteria on food processing facility surfaces. Accordingly, the novel, deposited probiotic isolates and the method of using such isolates as claimed herein are believed to be non-obvious over the combined teachings of Daeschel et al in view of Sulzer et al and DeLoach et al. Therefore, applicants respectfully request the withdrawal of the rejection of the claimed method as obvious over those references.

The claims as amended are believed to be in condition for allowance. Applicants respectfully request allowance of the claims, and passage of the application to issuance. If the Examiner has any questions or comments such that a conversation would speed prosecution of this application, the Examiner is invited to call the undersigned at (434) 220-2866.

Respectfully submitted,

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